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Effect of Stress on Selected Edible Plants

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ABSTRACT

Two edible plants *Lablab purpureus* of the Fabaceae and *Cucumis sativus* of the Cucurbitaceae have been studied by stress analysis. Salt stress is caused by excessive accumulation of salt in the soil, either directly because of salinization, or indirectly because of water loss. As a consequence, the soil water potential progressively decreases and eventually stopping the gradient of water flow from roots region to upper most part (shoot and leaf). In this paper, both edible plant seeds are allowed to different stress conditions (salt, dark and sugar). The different concentration of salt such as 10ppm, 25ppm, 50ppm, dark condition and 10ppm of sugar solution are used. Both plant seeds measurement was taken at the interval of 10 days. Generally first germination was seen within 3 days in a pot after sowing of seeds. In both plant, control pots showed normal growth of seeds. Treatments are shown the variation. Stress analysis is further confirmed by proline analysis. This paper revealed that stress analysis of entirely two different family members such as *Lablab purpureus* and *Cucumis sativus*

KEYWORDS: *Cucumis sativus, Lablab purpureus, Pproline Analysis, Salinity*

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INTRODUCTION

Salinity, Drought, flooding, high temperature, cold,, and nutrient availability are abiotic factors that have a significant impact on world agriculture and account for more than 50% reduction in average potential yields for most major food and fodder crops (Wang et al., 2003). These comprise mostly of high temperature (40%), salinity (20%), drought (17%), low temperature (15%) and other forms of stresses (Ashraf, 2008). Climate prediction models show increased occurrences of drought, flooding, salinity and high temperature spells during the crop growing periods (IPCC, 2008; Mittler and Blumwald, 2010) .

Seed germination is the first stage of the plant's life cycle, and is negatively affected by salinity (Feizi *et al.,* 2012). Several previous studies have shown that seed germination is extremely sensitive to salinity in most plant species (Heenan *et al*., 1988). Abbas et al. (2013) demonstrated that the percent germination, shoot and root length, and dry weight of rice (*Oryza sativa*) were reduced with increasing levels of NaCl. Similarly, in dicotyledonous cabbage (*Brassica oleracea*), seed germination and the growth of roots and buds were also inhibited under salt stress (Jamil et al., 2007). Therefore, salt tolerance at the germination stage is critical for the successful growth of plants in saline conditions.

Agricultural activities are functioning throughout India. They are mostly operated by smallholder farmers who run small mixed crop-livestock enterprises relying on low-inputs and rainfall only (Njarui and Wandera 2004; Njarui and Mureithi 2010). Since the rural areas offer only limited income activities apart from agriculture, agriculture still plays an important role for income generation and serves as a major livelihood activity of the mainly rural-based population (Maingi et al. 2001). Besides poor soil fertility (Dixon *et al.,* 2001; Macharia *et al.*, 2011), water is the most limiting factor for crop production in these areas. Rainfall is generally low and highly variable, leading to low crop yields and even complete crop failures (Gachimbi *et al.*, 2002; Muhammad *et al.*, 2010; Silvestri *et al.*, 2012). As a consequence of continous population growth and environmental restrictions, the pressure on land and resources is increasing the challenge of agricultural production in the semi-arid areas (Macharia *et al*., 2011).

Salinity is one of the most significant factors limiting crop productivity. Approximately 20% of the world's cultivated land and nearly half of all irrigated lands are affected by salinity (Zhu, 2001). Plant species and cultivars within a crop species differ greatly in their responses to salinity (Dasgan *et al*.2002) .

Plant cells decrease their osmotic potential by the accumulation of inorganic and organic solutes (Liu and Staden 2001). Proline is considered to be a compatible solute. It plays a major role in osmotic adjustment in potato and tomato under saline conditions (Claussen 2005). Salt stress resulted in a significant accumulation of free proline in the shoots of green gram (Misra and Gupta 2005) and mulberry (Kumar and Chauhan 2008). Rapid accumulation of free proline is a typical response to salt stress. When exposed to drought or a high salt content in the soil (both leading to water stress), many plants accumulate high amounts of proline, in some cases several times the sum of all the other amino acids (Mansour, 2000).

Proline has been found to protect cell membranes against salt injury (Mansour, 1998). Sultana et al. have suggested that proline accumulation in both salinized leaves and grains of rice plants is implicated in osmotic adjustment to salinity. In contrast, Lutts et al. have argued that proline accumulated in salt-stressed calluses had a negligible effect on osmotic adjustment and did not play a role in salt resistance in rice callus cultures. For many years, proline has been known to be involved in the response to a number of environmental stresses, particularly salt and drought stress. The accumulation of proline upon osmotic stress is well documented in a large number of different plant species (Yoshiba *et al.,* 1995, Mattioli *et al*., 2008). However, a general agreement on the precise role of proline in the response of plants to stress is still lacking and several hypotheses have been proposed on the significance of the accumulation of proline caused by stress (KaviKishor *et al*., 1995).

Lablab (*Lablab purpureus*) is one of those multipurpose legumes known for its great genetic diversity (Tefera 2006; Maass *et al*., 2010). Reported to thrive across a wide range of environmental conditions, its genetic diversity may have led to a high phenological plasticity, playing an important role in the development of drought resistance mechanisms (Subbarao *et al*., 1995; Turner *et al.,*2001). These mechanisms include the ability of lablab to grow deep tap roots enabling the plant to reach deep residual soil moisture (Duke *et al*., 2015; Smartt, 1990). Lablab is considered to cope better with drough conditions compared to some of the more widely grown legumes such as common beans (*Phaseolus vulgaris L*.) or cowpeas (*Vigna unguiculata L.* Walp.) (Maass *et al*., 2010).

Cucumber (*Cucumis sativus L*.) is one of the most popular vegetables under protected cultivation conditions. With the aggravation of soil secondary salinization, the yield of cucumber is accordingly reduced (Stepien and Klobus 2006). Thus, many efforts have been made to increase the salt tolerance of cucumber by researching the physiological responses of cucumber to salt stress. In the leaves of cucumber, NaCl stress decreases plant growth, chlorophyll content, the net photosynthetic rate, stomatal conductance, the transpiration rate and the quantum yield of PSII and increases the content of MDA and POD activities (Stepien and Klobus 2006; Wei *et al.,* 2004; Zhang *et al*., 2001) .

MATERIALS AND METHODS

Plant materials:

1. Lablab Purpureus:

Lablab purpureus is a species of bean in the family Fabaceae. It is native to Africa and it is cultivated throughout the tropics for food. English common names include bean/pea, lablab bean, Egyptian kidney bean, Indian bean. It is the only species in the monotypic genus Lablab. Local / Tamil name: Avarai

The plant is variable due to extensive breeding in cultivation, but in general, they are annual or short-lived perennial vines. The thick stems can reach six meters in length. The leaves are made up of three pointed leaflets each up to 15 centimeters long. They may be hairy on the undersides. The inflorescence is made up of racemes of many flowers. Some cultivars have white flowers, and others may have purplish or blue. The fruit is a legume pod variable in shape, size, and color. It is usually several centimeters long and bright purple to pale green. It contains up to four seeds. The seeds are white, brown, red, or black depending on the cultivar. The seed is about a centimeter long

2. Cucumis sativus

Cucumber (*Cucumis sativus*) is a widely-cultivated creeping vine plant in the Cucurbitaceae gourd family that bears cucumiform fruits, which are used as vegetables. There are three main varieties of cucumber—slicing, pickling and burpless/seedless—within which several cultivars have been created. The cucumber originates from South Asia, but now grows on most continents, as many different types of cucumber are traded on the global market. The fruit of typical cultivars of cucumber is roughly cylindrical, but elongated with tapered ends, and may be as large as 62 centimeters (24 in) long and 10 centimeters (4 in) in diameter. Cucumber fruits consist of 95% water. In botanical terms, the cucumber is classified as a pepo, a type of botanical berry with a hard outer rind and no internal divisions.

Soil preparation:

The good soil has been described as the capacity of soil to make nutrients available to the plants. Generally the soil mixture was prepared with sand, red soil and garden soil (1:2:1). The soil was sun dried for sterilization after mixing of soil. The soil pH was adjusted to 7 before sowing the seeds in pots.

Treatment details for both plants:

Treatment I: Salt solution (10ppm)

Treatment II: Salt solution (25ppm)

Treatment III: Salt solution (50ppm)

Treatment IV: Sugar solution (10ppm)

Treatment V: Dark condition

Place/Location:

The healthy seeds of two edible plants are collected and sowed in different pots and kept in the Green house (Department of Botany), The American College, Madurai.

Optimal temperature and Moisture for Green house

Optimal temperature: Between 17-27°C

Moisture: 50-70% relative humidity

- Seeds are sowed in pots.
- Add different Conc. of salt and sugar solution to the pots at the intervals of 10 days.
- The pots are labelled.
- One set of pot put in a Dark condition (in each)
- Additionally one pot is kept as control in each plant.
- The seed growth in each pots are monitored and measured at 10days interval

Proline Analysis

By homogenizing 0.5 gm of fresh plant material in 10 ml of 3% aqueous sulphosalicyclic acid, the extract was made. The homogenate is filtered through Whatman No. 2 filter paper. 2 ml of filtrate is taken in a test tube and 2 ml of glacial acetic acid and 2 ml acid-ninhydrin are added in a sequence. The mixture is heated it in the boiling water-bath for 1 h. The reaction is stopped by placing the tube in ice-bath. 4 ml toluene is added to the reaction mixture and stir well for 20-30 sec. The toluene layer is separated and warm to room temperature. The red colour intensity is measured at 520

nm. A series of standard with pure proline in a similar way is made and prepare a standard curve. Thus the amount of proline in the test sample (value) is compared with the standard proline curve.

Calculation:

Express the proline content on fresh-weight-basis as follows:

$$\text{mmoles per g tissue} = \frac{\text{mg proline/mL x mL toluene}}{115.5} \times \frac{5}{\text{gm sample}}$$

(*where 115.5 is the molecular weight of proline)

RESULT AND DISCUSSION

Table 1 showed that the various treatment of L*ablab purpureus* with measurements. Every pot contained 10 numbers of seeds. Average growth of the seed was taken as single value. After 10 days interval the measurement was taken and recorded for compare with other treatments. Generally first germination was seen within 3 days in a pot after sowing of seeds. L*ablab purpureus* control pots showed normal growth of seeds that is 1.5cm, 8.2cm, 13cm and14.4cm respectively. In treatment 1 showed that there is no growth within three days after sowing of seeds. In first interval it showed the germination of seeds in with limited height that is measured as 3cm. Then measurement was taken in 2nd, 3rd interval as 7.2cm and 8cm respectively. There is no growth occurred in Treatment II and Treatment III (fig-1). Treatment IV has equal seed growth of the control that is measured as 1.2 cm, 9cm, 14 cm and 15.5 cm respectively (fig-3). Treatment V showed high growth of seedlings while compare with control. The measurement was 1.5cm, 9.5cm, 11cm respectively 20.1 cm (fig-2).

Table 1 Various treatment of Lablab purpureus with measurements

S. No	Plant with Treatment	Date of Measurement (Average in cm)			
		20.01.21	30.1.21	09.02.21	18.02.21
1	Lablab Purpureus (Control)	1.5cm	8.2cm	13cm	14.4cm
2	Lablab Purpureus(Salt 10ppm) (T I)	-	3cm	7.2cm	8cm
3	Lablab Purpureus (Salt 25ppm) (T II)	-	-	-	-
4	Lablab Purpureus (Salt 50ppm) (T III)	-	-	-	-
5	Lablab Purpureus(Sugar 10ppm) (T IV)	1.2cm	9cm	14cm	15.5cm
6	Lablab Purpureus(Dark) (T V)	1.5cm	9.5cm	11cm	20.1cm

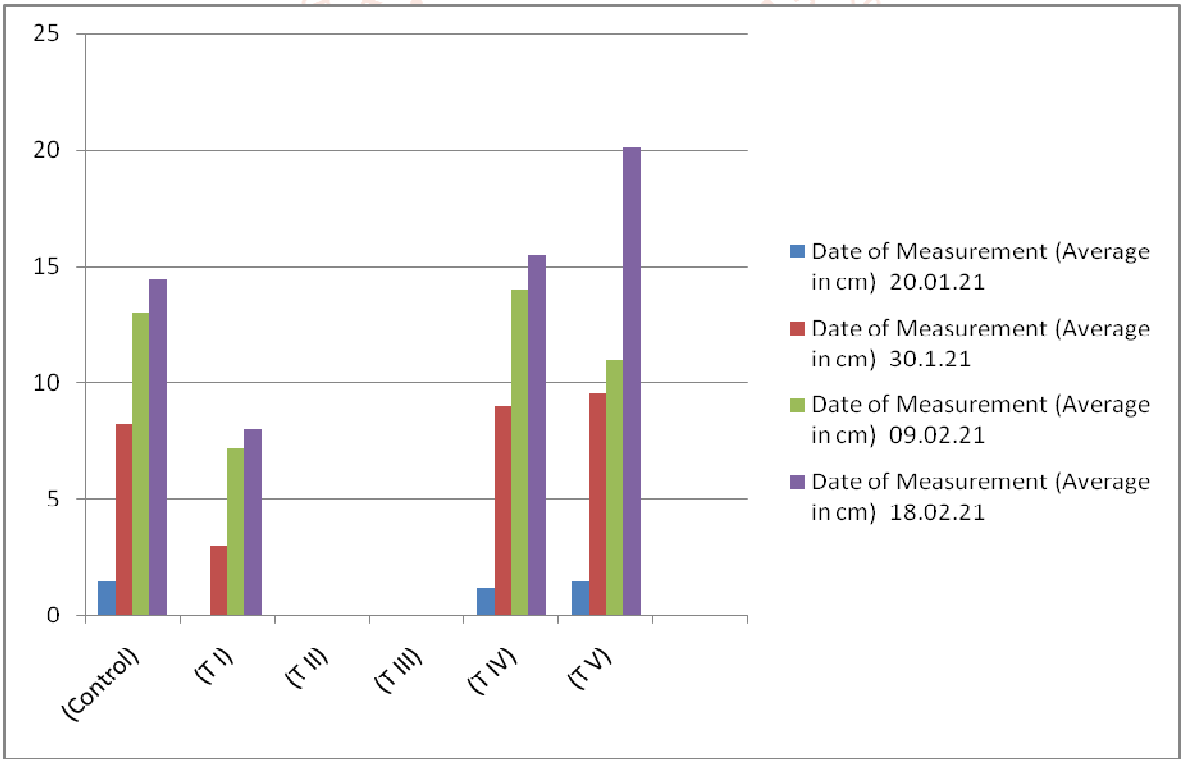

Table 2 Various treatment of Cucumis sativus with measurements

S. No	Plant with Treatment	Date of Measurement (Average in cm)			
		20.01.21	30.1.21	9.2.21	18.2.21
1	Cucumis sativus (Control)	0.6cm	4.8cm	8.2cm	11.2cm
2	Cucumis sativus (Salt 10ppm) TI	-	3.2cm	5.2cm	7.2cm
3	Cucumis sativus (Salt 25ppm) TII	-	-	-	-
4	Cucumis sativus (Salt 50ppm) TIII	-	-	-	-
5	Cucumis sativus (Sugar 10ppm) TIV	1cm	6cm	8.5cm	12cm
6	Cucumis sativus (Dark) TV	0.9cm	5.5cm	10.2cm	17.2cm

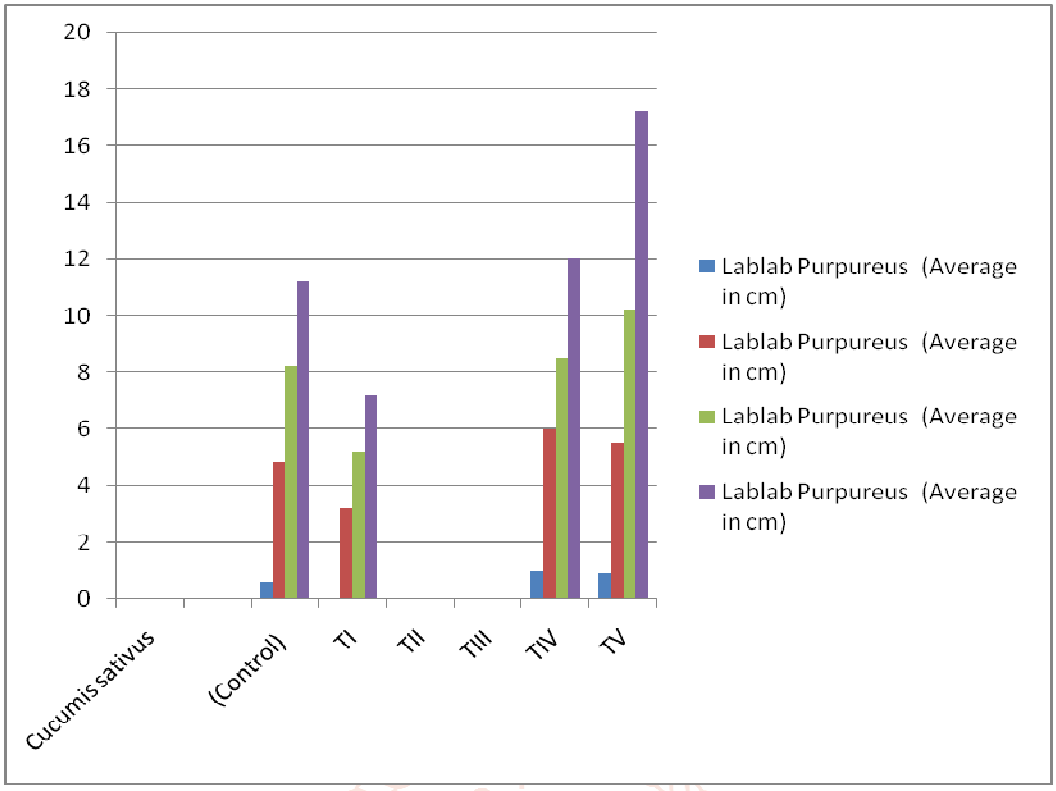

Table 2 showed that the various treatment of *Cucumis sativus* with measurements. Every pot contained 10 numbers of seeds. Average growth of the seed was taken as single value. After 10 days interval the measurement was taken and recorded for compare with other treatments. Generally first germination was seen within 3 days in a pot after sowing of seeds. *Cucumis sativus* control pots showed normal growth of seeds that is 0.6cm, 4.8cm, 8.2cm and11.2cm respectively (fig-5). In treatment 1 showed that there is no growth within three days after sowing of seeds. In first interval it showed the germination of seeds in with limited height that is measured as 3.2cm. Then measurement was taken in 2nd, 3rd interval as 5.2cm and 7.2cm respectively(fig-6). There is no growth occurred in Treatment II and Treatment III (fig-4). Treatment IV has equal seed growth of the control that is measured as 1cm, 6cm, 8.5cm and 12cm respectively (fig-3). Treatment V showed high growth of seedlings while compare with control. The measurement was 0.9cm, 5.5cm,10.2cm respectively 17.2cm (fig-2).

Figure1: *Lablab Purpureus* (Control, Treatment I and Treatment III)

Figure 2: *Lablab Purpureus* (Treatment V) Figure 3: *Lablab Purpureus* (Treatment IV)

Figure 4: *Cucumis sativus* (Control, Treatment II and Treatment III)

Figure 5: *Cucumis sativus* (Control) **Figure 6: *Cucumis sativus* (Treatment I)** **Figure 7: *Cucumis sativus* (Treatment IV)**

Table 3 Proline analysis with plant (leaf only)

S. No.	Plant	Treatment type	Amount of proline present in fresh leaf (µmoles)
1	Lablab Purpureus	Control	0.10
		Treatment I (Salt 10ppm)	0.45
2	Cucumis sativus	Control	0.13
		Treatment I (Salt 10ppm)	0.47

Fig 8. Estimation of proline in fresh leaf of *Lablab Purpureus* and *Cucumis sativus:*

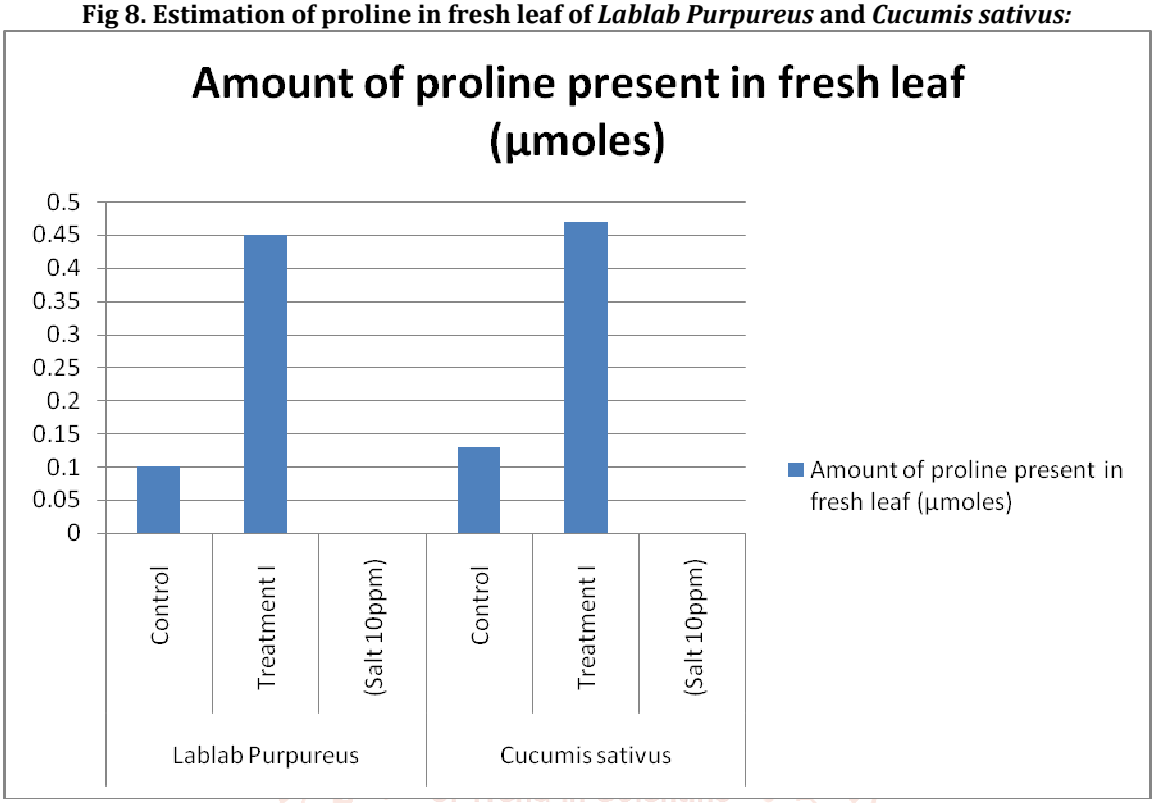

Table 3 and figure 8 showed that the amount of proline accumulated in the fresh leaf materials. In normal condition (unstressed), the fresh leaf of *Lablab Purpureus* (0.5mg) contained 0.10µmoles of proline where as treatment I of *Lablab Purpureus contained* 0.45 µmoles of proline.The fresh leaf of *Cucumis sativus* (0.5mg) contained 0.13 µmoles of proline where as treatment I of *Cucumis sativus contained* 0.47 µmoles of proline.

Table 1 and table 2 are showed that the various treatment of L*ablab purpureus and Cucumis sativus* with measurements. Every pot contained 10 numbers of seeds. Average growth of the seed was taken as single value. After 10 days interval the measurement was taken and recorded for compare with other treatments. Salt concentration (10ppm) in both plants are showing minimum growth while compare to the normal growth. Similarly salinity stress affects the development and underlying mechanisms such as seed germination, seedling growth and vigor, vegetative growth, flowering, and fruit set. A general decline in physical parameters in Lablab during salt stress in time- and concentration-dependent manner indicated that the salt stress above 100 mM significantly affects the growth potential. The decrease in growth can be attributed to the reduced cell elongation resulting from decreased turgor, cell volume, and cell growth, as has been observed by Boyer (1988). Contrary to salinity-stressed soybean (Murat *et al*., 2008), Lablab seedlings showed a moderate reduction in fresh and dry weights up to 300 mM. Observed changes in shoot and root lengths of Lablab were similar to those of salt-tolerant Lucerne (Rogers *et al*. 2003), and salt- and temperature stressed French bean (Nagesh and Devaraj 2008). Nevertheless, salinity influenced Lablab shoot growth more negatively than root, similar to Medicago citrina (Sibole *et al.,*2005), indicating the moderate tolerance of the plant. Reduced effect on the root growth could be due to expenditure of more photosynthetic energy on root growth in search of water and/or reducing water loss, thus maintaining higher water relations.

Similarly combination of NaCl, Na2CO3 and K2SO4 salts were used to induce salt stress artificially. Increased levels of salt concentration resulted in progressive reduction in germination (78.47, 43.67 and 24.90%), number of leaves (19.26, 10.56 and 6.30%), survivability (93.77, 59.79 and 39.25%), vine length (88.99, 49.07 and 28.92 cm) and fruit yield per vine (1.10, 0.62 and 0.29 kg) ; while increased the affected leaves (28.73, 68.92 and 82.59%) and defoliation (13.39, 59.74 and 74.83%) (Tiwari et al., 2013).

Sodium content, Na+–K+ ratio, proline, reducing sugars, phenol and yield reduction (%) increased significantly as the salt stress increased. Potassium, chlorophyll, membrane stability index and fruit yield decreased significantly under salt stress in all genotypes of cucumber. However, the genotypes CRC-8, CHC-2 and G-338 showed lower accumulation of sodium, lesser depletion of potassium, lower Na+–K+ ratio and higher accumulation of proline, reducing sugars, phenols, better membrane stability and lower yield reduction (%) under salt stress, while CH-20 and DC-1 were sensitive to salt stress (Tiwari et al., 2010). In our study, both plants are also more sensitive the salt condition. It leads to reduction in growth and development. Salt condition to production of proline accumulation in fresh materials (leaf)

Table 3 and figure 8 showed that the amount of proline accumulated in the fresh leaf materials when they are exposed salt conditions. Because previous studies are

explained that the proline is a non enzymatic molecule is known to accumulate under different types of abiotic stress. Plants accumulate several kinds of osmolytes such as proline, glycine betaine and soluble sugars under stress condition. Proline has been implicated as antistress organic molecule, in some higher plants (Greenway and Munns, 1980) and it is known to accumulate in response to environmental stress (Aspinall and Paleg, 1981). In our study, proline accumulated in fresh material and that was estimated (Table 3 and fig 3). It is quite interesting to note that the *Lablab Purpureus* and *Cucumis sativus* plants are grown in salt concentration(10ppm). That showed some amount of free proline accumulation. It has also been reported that the plants grown under stress condition exhibit a remarkable increase in proline content in some legumes (Saralabai and Vivekanandan, 1995). Proline accumulation was normally observed during stress condition.

Increased accumulation of proline is to maintain intercellular osmoticum during stress condition. The higher magnitude of proline accumulation may help plants to tolerate the degradation by maintaining cell turgidity as recorded earlier by Sivakumar *et al*. (2013) and may protect plants against induced damage. The accumulation of proline content under stress may be due to increased synthesis of protein bound proline (Krishnamurthy *et al*., 2010) .

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